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=> s antibody

L1 2180818 ANTIBODY

=> s l1 and nitrodylated protein

L2 0 L1 AND NITRODYLATED PROTEIN

=> s l1 and nitrosylated protein

L3 6 L1 AND NITROSYLATED PROTEIN

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PROCESSING COMPLETED FOR L3
L4 2 DUP REMOVE L3 (4 DUPLICATES REMOVED)

=> d l4 1-2 cbib abs

L4 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2001 ACS
1998:485093 Document No. 129:121648 **Antibodies** specifically recognizing a **nitrosylated protein**, method of preparation, and therapeutic and diagnostic use. Chagnaud, Jean-Luc; Geffard, Michel; Veyret, Bernard; Vincendeau, Philippe (Centre National de la Recherche Scientifique (CNRS), Fr.). PCT Int. Appl. WO 9829452 A1 19980709, 143 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (French). CODEN: PIXXD2.
APPLICATION: WO 1997-FR2412 19971223. PRIORITY: FR 1996-16207 19961230.
AB Polyclonal and monoclonal **antibodies** are provided which specifically recognize a **nitrosylated protein**, and, more particularly, a NO carrier, e.g. albumin. Also provided are immunogens for prepg. the **antibodies** and the pharmaceutical compns. contg. them. Further provided is a method using the **antibodies** for detecting in vitro **nitrosylated proteins** in a biol. sample.

L4 ANSWER 2 OF 2 MEDLINE DUPLICATE 1
1998019329 Document Number: 98019329. PubMed ID: 9353418. Nitrosylated bovine serum albumin derivatives as pharmacologically active nitric oxide congeners. Ewing J F; Young D V; Janero D R; Garvey D S; Grinnell T A. (NitroMed, Inc., Bedford, Massachusetts 01730, USA.) JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1997 Nov) 283 (2) 947-54. Journal code: JP3; 0376362. ISSN: 0022-3565. Pub. country: United States. Language: English.
AB Although nitrosothiols have been suggested to act as regulators of cell (patho)physiology, little is known about the pharmacology of **nitrosylated proteins** as nitric oxide (NO.) congeners. We describe the molecular consequences of nitrosylating bovine serum albumin (BSA) at multiple specific sites and demonstrate that the product S-nitrosoproteins exert NO.-like activity. The content of nucleophilic nitrosylation sites (i.e., free sulfhydryl groups) in native BSA was

increased by either reduction with dithiothreitol or thiolation with N-acetylhomocysteine. Fourteen moles of nitrogen monoxide (NO)/mol BSA equivalent were then selectively positioned on either the endogenous sulfhydryl groups of reduced BSA or the homocysteine moieties of thiolated BSA, respectively. Each resulting S-nitrosoprotein adduct was an oligomeric mixture across the >2000 kDa to approximately 66 kDa molecular mass range. The BSA-derived S-nitrosoproteins were immunoreactive with **antibodies** against native BSA but evidenced compromised long-chain fatty acid binding. Both types of BSA-derived S-nitrosoproteins suppressed human coronary artery smooth muscle cell proliferation to a similar degree (IC50 approximately 70 microM NO. equivalents) and were significantly more effective antiproliferative agents than a standard NO. donor, DETA NONOate. Antiproliferative bioactivity reflected the NO functionalities carried by each protein, but was independent of molecular mass of the nitrosylated BSA adducts. These data exemplify the rational design and characterization of protein-based S-nitrosothiols as NO. congeners and suggest that such agents could have therapeutic potential as NO delivery systems.

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L1 2180818 S ANTIBODY
L2 0 S L1 AND NITRODYLATED PROTEIN
L3 6 S L1 AND NITROSYLATED PROTEIN
L4 2 DUP REMOVE L3 (4 DUPLICATES REMOVED)

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L5 7 L1 AND NITROSYLATED ALBUMIN

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L6 3 DUP REMOVE L5 (4 DUPLICATES REMOVED)

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L6 ANSWER 1 OF 3 MEDLINE DUPLICATE 1
2000105692 Document Number: 20105692. PubMed ID: 10637125. Mechanism of extracellular thiol nitrosylation by N(2)O(3) produced by activated macrophages. Gobert A P; Vincendeau P; Mossalayi D; Veyret B. (Laboratoire de Physique des Interactions Ondes-Matiere (PIOM), UMR 5501, CNRS, ENSCPB, Talence, France.) NITRIC OXIDE, (1999 Dec) 3 (6) 467-72. Journal code: C5F; 9709307. ISSN: 1089-8603. Pub. country: United States. Language: English.

AB Reactive nitrogen intermediates are synthesized by activated macrophages. These molecules, and nitrous anhydride (N(2)O(3)) in particular, are known to be potent nitrosylating species. We investigated the role of macrophage-derived N(2)O(3) in extracellular nitrosylation. We used dilution experiments to demonstrate the intracellular production of N(2)O(3) and its export into the extracellular medium, with a rate constant k(ex) = 6.8 x 10(6) M s(-1). The kinetics of the competition between extracellular hydrolysis of N(2)O(3) and its reaction with added glutathione were also studied. We obtained a value of the rate constant k(GSH) for the latter reaction of 4.4 x 10(7) M(-1) s(-1), consistent with earlier determinations in cell-free systems. The implications of these results in human albumin nitrosylation were investigated. **Nitrosylated albumin** was detected in activated macrophages supernatants using an anti-NO-acetylated cysteine **antibody**. It was estimated that 10% of N(2)O(3) produced by activated cells participate in extracellular nitrosylation. N(2)O(3) thus appears to be a new effector molecule of the immune system, as an agent for the nitrosylation of albumin, the main nitric oxide carrier in vivo. Copyright 1999 Academic Press.

L6 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS

1997:749550 Document No. 128:70584 Nitrosylated bovine serum albumin derivatives as pharmacologically active nitric oxide congeners. Ewing, James F.; Young, Delano V.; Janero, David R.; Garvey, David S.; Grinnell, Todd A. (NitroMed, Inc., Bedford, MA, USA). J. Pharmacol. Exp. Ther., 283(2), 947-954 (English) 1997. CODEN: JPETAB. ISSN: 0022-3565. Publisher: Williams & Wilkins.

AB Although nitrosothiols have been suggested to act as regulators of cell (patho)physiol., little is known about the pharmacol. of nitrosylated proteins as nitric oxide (NO.cntdot.) congeners. We describe the mol. consequences of nitrosylating bovine serum albumin (BSA) at multiple specific sites and demonstrate that the product S-nitrosoproteins exert NO.cntdot.-like activity. The content of nucleophilic nitrosylation sites (i.e., free sulfhydryl groups) in native BSA was increased by either redn. with dithiothreitol or thiolation with N-acetylhomocysteine. Fourteen moles of nitrogen monoxide (NO)/mol BSA equiv. were then selectively positioned on either the endogeneous sulfhydryl groups of reduced BSA or the homocysteine moieties of thiolated BSA, resp. Each resulting S-nitrosoprotein adduct was an oligomeric mixt. across the >2000 kDa to .apprxeq.66 kDa mol. mass range. The BSA-derived S-nitrosoproteins were immunoreactive with **antibodies** against native BSA but evidenced compromised long-chain fatty acid binding. Both types of BSA-derived S-nitrosoproteins suppressed human coronary artery smooth muscle cell proliferation to a similar degree (IC50 .apprxeq. 70 NO.cntdot. equiv.) and were significantly more effective antiproliferative agents than a std. NO.cntdot. donor, DETA NONOate. Antiproliferative bioactivity reflected the NO functionalities carried by each protein, but was independent of mol. mass of the nitrosylated BSA adducts. These data exemplify the rational design and characterization of protein-based S-nitrosothiols as NO.cntdot. congeners and suggest that such agents could have therapeutic potential as NO delivery systems.

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2001 ACS

1997:13532 Document No. 126:58589 Albumin nitrosylated by activated macrophages possesses antiparasitic effects neutralized by anti-NO-acetylated-cysteine **antibodies**. Mnaimneh, Sanie; Geffard, Michel; Veyret, Bernard; Vincendeau, Philippe (Parasitology Lab., Univ. Bordeaux II, Bordeaux, Fr.). J. Immunol., 158(1), 308-314 (English) 1997. CODEN: JOIMA3. ISSN: 0022-1767. Publisher: American Association of Immunologists.

AB Activated macrophages exert an L-arginine-dependent cytostatic effect on the extracellular parasite, Trypanosoma musculi. This effect is not obsd. in the absence of albumin in the culture medium but is restored by the addn. of albumin, indicating the presence of an albumin-nitric oxide (NO) adduct acting as an effector mol. Since L-cysteine represents a privileged target for NO, an immunochem. approach was performed using an acetylated-cysteine-BSA conjugate. This conjugate was nitrosylated using sodium nitrite as a NO donor. Binding of NO to the conjugated haptens was assayed using spectrophotometry. It was completely abolished by mercuric chloride, confirming the presence of an S-NO bond. Polyclonal Abs were obtained after immunizing rabbits with S-nitroso-acetylated-cysteine (NO-ac-Cys) conjugates. Using the ELISA method, Ab avidity and specificity were detd. by competition expts. between NO-ac-Cys-conjugated compds. and other nitrosylated or non-nitrosylated compds. The resulting cross-reactivity ratios showed that conjugated NO-ac-Cys-BSA was the best recognized compd. These Ab were used for an in vitro study of the kinetics of NO-derived compds. from activated murine macrophages. Anti-NO-ac-Cys Ab inhibited the antimicrobial effect of activated macrophages on the extracellular parasite, T. musculi. Moreover, the L-arginine-dependent antiparasitic activity of supernatants from Calmette-Guerin bacillus-activated macrophages required the presence of albumin and was also inhibited by anti-NO-ac-Cys Ab, showing the effector role of S-nitroso-albumin.

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L7 1358 (CHAGNAUD J?/AU OR GEFFARD M?/AU OR VEYRET B?/AU OR VINCENDEAU P/AU)

=> s l7 antibody

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L8 565 L7 AND ANTIBODY

=> s l8 and nitrosylated protein

L9 1 L8 AND NITROSYLATED PROTEIN

=> d l9 cbib abs

L9 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
1998:485093 Document No. 129:121648 **Antibodies** specifically recognizing a **nitrosylated protein**, method of preparation, and therapeutic and diagnostic use. **Chagnaud, Jean-Luc; Geffard, Michel; Veyret, Bernard;** Vincendeau, Philippe (Centre National de la Recherche Scientifique (CNRS), Fr.). PCT Int. Appl. WO 9829452 A1 19980709, 143 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (French). CODEN: PIXXD2. APPLICATION: WO 1997-FR2412 19971223. PRIORITY: FR 1996-16207 19961230.

AB Polyclonal and monoclonal **antibodies** are provided which specifically recognize a **nitrosylated protein**, and, more particularly, a NO carrier, e.g. albumin. Also provided are immunogens for prepg. the **antibodies** and the pharmaceutical compns. contg. them. Further provided is a method using the **antibodies** for detecting in vitro **nitrosylated proteins** in a biol. sample.

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L10 202 DUP REMOVE L8 (363 DUPLICATES REMOVED)

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L11 0 L10 AND ANTI-NITROSYLATED ALBUMIN

=> s l10 albumin

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L13 ANSWER 1 OF 24 MEDLINE
2000105692 Document Number: 20105692. PubMed ID: 10637125. Mechanism of extracellular thiol nitrosylation by N(2)O(3) produced by activated macrophages. Gobert A P; **Vincendeau P;** Mossalayi D; **Veyret B.** (Laboratoire de Physique des Interactions Ondes-Matiere (PIOM), UMR 5501, CNRS, ENSCPB, Talence, France.) NITRIC OXIDE, (1999 Dec) 3 (6) 467-72. Journal code: C5F; 9709307. ISSN: 1089-8603. Pub. country: United States. Language: English.

AB Reactive nitrogen intermediates are synthesized by activated macrophages. These molecules, and nitrous anhydride (N(2)O(3)) in particular, are known

to be potent nitrosylating species. We investigated the role of macrophage-derived N(2)O(3) in extracellular nitrosylation. We used dilution experiments to demonstrate the intracellular production of N(2)O(3) and its export into the extracellular medium, with a rate constant k(ex) = 6.8 x 10(6) M s(-1). The kinetics of the competition between extracellular hydrolysis of N(2)O(3) and its reaction with added glutathione were also studied. We obtained a value of the rate constant k(GSH) for the latter reaction of 4.4 x 10(7) M(-1) s(-1), consistent with earlier determinations in cell-free systems. The implications of these results in human **albumin** nitrosylation were investigated. Nitrosylated **albumin** was detected in activated macrophages supernatants using an anti-NO-acetylated cysteine **antibody**. It was estimated that 10% of N(2)O(3) produced by activated cells participate in extracellular nitrosylation. N(2)O(3) thus appears to be a new effector molecule of the immune system, as an agent for the nitrosylation of **albumin**, the main nitric oxide carrier in vivo.
Copyright 1999 Academic Press.

=> d l13 2-24 cbib abs

L13 ANSWER 2 OF 24 CAPLUS COPYRIGHT 2001 ACS
1998:485093 Document No. 129:121648 **Antibodies** specifically recognizing a nitrosylated protein, method of preparation, and therapeutic and diagnostic use. **Chagnaud, Jean-Luc; Geffard, Michel ; Veyret, Bernard;** Vincendeau, Philippe (Centre National de la Recherche Scientifique (CNRS), Fr.). PCT Int. Appl. WO 9829452 A1 19980709, 143 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (French). CODEN: PIXXD2.
APPLICATION: WO 1997-FR2412 19971223. PRIORITY: FR 1996-16207 19961230.
AB Polyclonal and monoclonal **antibodies** are provided which specifically recognize a nitrosylated protein, and, more particularly, a NO carrier, e.g. **albumin**. Also provided are immunogens for prepg. the **antibodies** and the pharmaceutical compns. contg. them. Further provided is a method using the **antibodies** for detecting in vitro nitrosylated proteins in a biol. sample.

L13 ANSWER 3 OF 24 MEDLINE
97131703 Document Number: 97131703. PubMed ID: 8977204. **Albumin** nitrosylated by activated macrophages possesses antiparasitic effects neutralized by anti-NO-acetylated-cysteine **antibodies**. Mnaimneh S; **Geffard M; Veyret B; Vincendeau P.**
(Parasitology Laboratory, University of Bordeaux II, France.) JOURNAL OF IMMUNOLOGY, (1997 Jan 1) 158 (1) 308-14. Journal code: IFB; 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB Activated macrophages exert an L-arginine-dependent cytostatic effect on the extracellular parasite, Trypanosoma musculi. This effect is not observed in the absence of **albumin** in the culture medium but is restored by the addition of **albumin**, indicating the presence of an **albumin**-nitric oxide (NO) adduct acting as an effector molecule. Since L-cysteine represents a privileged target for NO, an immunochemical approach was performed using an acetylated-cysteine-BSA

conjugate. This conjugate was nitrosylated using sodium nitrite as a NO donor. Binding of NO to the conjugated haptens was assayed using spectrophotometry. It was completely abolished by mercuric chloride, confirming the presence of an S-NO bond. Polyclonal Abs were obtained after immunizing rabbits with S-nitroso-acetylated-cysteine (NO-ac-Cys) conjugates. Using the enzyme-linked immunosorbent assay method, Ab avidity and specificity were determined by competition experiments between NO-ac-Cys-conjugated compounds and other nitrosylated or non-nitrosylated compounds. The resulting cross-reactivity ratios showed that conjugated NO-ac-Cys-BSA was the best recognized compound. These Ab were used for an in vitro study of the kinetics of NO-derived compounds from activated murine macrophages. Anti-NO-ac-Cys Ab inhibited the antimicrobial effect of activated macrophages on the extracellular parasite, T. musculi. Moreover, the L-arginine-dependent antiparasitic activity of supernatants from Calmette-Guerin bacillus-activated macrophages required the presence of **albumin** and was also inhibited by anti-NO-ac-Cys Ab, showing the effector role of S-nitroso-**albumin**.

L13 ANSWER 4 OF 24 MEDLINE

96235163 Document Number: 96235163. PubMed ID: 8642067. Circulating **antibodies** directed against conjugated fatty acids in sera of patients with multiple sclerosis. Boullerne A; Petry K G; **Geffard M.** (INSERM U394 Neurobiologie integrative, Bordeaux, France.) JOURNAL OF NEUROIMMUNOLOGY, (1996 Mar) 65 (1) 75-81. Journal code: HSO; 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB Using an adapted ELISA assay, we have tested sera from multiple sclerosis (MS) patients for **antibodies** directed against ten fatty acids conjugated to bovine serum **albumin**. In serum samples from 68 MS patients and 20 patients suffering from rheumatoid arthritis (RA), a significant **antibody** titer elevation to the ten tested fatty acids was found when compared to sera of 40 healthy subjects and 82 patients with other neurological and autoimmune diseases. G-200 purified IgM of MS patients reacted specifically with the aliphatic chains with an avidity of 3 x 10(-7) M. These results suggest that in MS and RA, autoepitopes on cell membranes that are normally hidden from the immune system become immunogenic. This may arise because of previous membrane disruption by oxidative processes.

L13 ANSWER 5 OF 24 MEDLINE

95273008 Document Number: 95273008. PubMed ID: 7753478. Molecular detection of methionine in rat brain using specific **antibodies**. Amara A; Coussemacq M; **Geffard M.** (Laboratoire d'Immunologie et Pathologie, Universite de Bordeaux II, France.) NEUROSCIENCE LETTERS, (1995 Feb 13) 185 (3) 147-50. Journal code: N7N; 7600130. ISSN: 0304-3940. Pub. country: Ireland. Language: English.

AB In order to study the localization of methionine in rat brain, an immunological approach was developed by raising **antibodies** directed against this amino acid. Methionine was conjugated to bovine serum **albumin** (BSA) or human serum **albumin** (HSA) via glutaraldehyde. The conjugates were then reduced by sodium borohydride and injected alternately into rabbits. **Antibody** affinity and specificity were evaluated using an adapted ELISA method, by competition experiments between conjugated methionine and related conjugated compounds, pre-incubated with anti-methionine **antibodies** diluted at 1/20,000. The resulting cross-reactivity ratios, calculated at half-displacement, showed that glutaraldehyde-methionine conjugate

(methionine-G-BSA) was the best recognized compound. Non-reduced methionine conjugate (methionine=G=BSA) and the related-conjugated molecules such as homocysteine, homocysteic acid, cysteine, cystathionine and glutamate were not recognized at all. **Antibodies** to methionine were directed against a glutaraldehyde-methionine epitope and their very high affinity and specificity made them reliable tools for molecular detection of methionine in rat brain. Using purified **antibodies** diluted at 1/20,000, motoneurons were found to be the most methionine-immunoreactive cell bodies in glutaraldehyde-fixed rat brain sections.

L13 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2001 ACS
1995:331098 Document No. 122:103925 Utilization of molecules recognized by autoantibodies of human serum for the diagnosis or treatment of AIDS. **Geffard, Michel** (Fr.). PCT Int. Appl. WO 9427151 A1 19941124, 36 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (French). CODEN: PIXXD2. APPLICATION: WO 1994-FR597 19940519. PRIORITY: FR 1993-6071 19930519.
AB The invention relates to the utilization of one or more mols. (e.g., fatty acids, cysteine and its derivs., 7-dehydrocholesterol, other cholesterol derivs., etc.) capable of being recognized by autoantibodies present in the serum of patients infected by the HIV-type virus, and to the implementation of methods of diagnosis in vitro of the infection of a person by the HIV-type virus, and to the prepn. of drugs intended for the treatment of AIDS.

L13 ANSWER 7 OF 24 MEDLINE
92193826 Document Number: 92193826. PubMed ID: 1372343. First characterization of 6-hydroxytryptamine in the rat midbrain by using specific **antibodies**. Dabadie H; **Geffard M**; Charrier M C; Locuratolo D; Berrier C; Jacquesy J C. (Laboratoire d'Immunologie et de Pathologie, INSERM CJF 88-13, Universite de Bordeaux II, France.) JOURNAL OF NEUROCHEMISTRY, (1992 Apr) 58 (4) 1292-9. Journal code: JAV; 2985190R.
ISSN: 0022-3042. Pub. country: United States. Language: English.
AB The visualization of serotonin, 5-methoxytryptamine, and tryptamine in the rat midbrain has been made possible by the development of **antibodies** raised against these conjugated molecules. It has been suggested that 6-hydroxytryptamine (6-HT) might also be a neurotransmitter in this region. To test this hypothesis, 6-HT was synthesized and **antibodies** were raised in the rabbit. The high avidity (IC50 = 5 x 10(-9) M) and specificity [cross-reactivity ratio between 6-HT-glutaraldehyde (G)-bovine serum **albumin** (BSA) and 5-HT-G-BSA, the most immunoreactive compound, was 1,500] rendered these **antibodies** reliable tools for specific molecular detection of 6-HT in the G-fixed tissues. In the dopaminergic region, 6-HT immunoreactivity was noted in the substantia nigra but was particularly intense in the red nuclei, where it seems to be localized in the magnocellular division in the form of large 6-HT neurons. In contrast, there were few 6-HT neurons in the raphe nuclei. Thus, 6-HT may be a new putative neurotransmitter existing in the red nuclei, in addition to the other neurotransmitters already described in this region, in the nigro-rubral pathway, and in the rubral projection from the dorsal raphe nuclei. 6-HT is possibly

implicated in motor control and might exert hallucinogenic properties as do other 6-hydroxylated indoleamines.

L13 ANSWER 8 OF 24 MEDLINE
91190153 Document Number: 91190153. PubMed ID: 2012621. **Antibody** responses of mice exposed to low-power microwaves under combined, pulse-and-amplitude modulation. **Veyret B**; Bouthet C; Deschaux P; de Seze R; **Geffard M**; Joussot-Dubien J; le Diraison M; Moreau J M; Caristan A. (Laboratoire de Bioelectromagnetisme de l'Ecole Pratique des Hautes Etudes: ENSCPB.) BIOELECTROMAGNETICS, (1991) 12 (1) 47-56. Journal code: 9Z7; 8008281. ISSN: 0197-8462. Pub. country: United States. Language: English.
AB Irradiation by pulsed microwaves (9.4 GHz, 1 microsecond pulses at 1,000/s), both with and without concurrent amplitude modulation (AM) by a sinusoid at discrete frequencies between 14 and 41 MHz, was assessed for effects on the immune system of Balb/C mice. The mice were immunized either by sheep red blood cells (SRBC) or by glutaric-anhydride conjugated bovine serum **albumin** (GA-BSA), then exposed to the microwaves at a low rms power density (30 microW/cm2; whole-body-averaged SAR approximately 0.015 W/kg). Sham exposure or microwave irradiation took place during each of five contiguous days, 10 h/day. The **antibody** response was evaluated by the plaque-forming cell assay (SRBC experiment) or by the titration of IgM and IgG **antibodies** (GA-BSA experiment). In the absence of AM, the pulsed field did not greatly alter immune responsiveness. In contrast, exposure to the field under the combined-modulation condition resulted in significant, AM-frequency-dependent augmentation or weakening of immune responses.

L13 ANSWER 9 OF 24 MEDLINE
92003561 Document Number: 92003561. PubMed ID: 2129868. Monoclonal anti-conjugated azelaic acid **antibody** production: application to multiple sclerosis. **Chagnaud J L**; Gosset I; Brochet B; Audhuy S; **Geffard M**. (Laboratoire d'Immunologie et Pathologie, CJF 88-13 INSERM, Universite de Bordeaux II, France.) NEUROREPORT, (1990 Oct) 1 (2) 141-4. Journal code: A6M; 9100935. ISSN: 0959-4965. Pub. country: ENGLAND: United Kingdom. Language: English.
AB We have previously reported the existence of anti-conjugated azelaic acid (Aze A) **antibodies** in the serum of patients with multiple sclerosis (MS). In order to demonstrate the specificity of these **antibodies**, we have produced a monoclonal **antibody** directed against Aze A conjugated by an acylation reaction to a protein. In competition experiments, with ELISA method, we demonstrated that a part of the **antibodies**, raised in rabbit after immunization by human immunoglobulins (Ig) of MS patients, recognized the antigen-combining site of our monoclonal anti-conjugated Aze A **antibody**. These results clearly demonstrate that a part of human Ig obtained from sera of MS patients shared common idiotopes with mouse monoclonal **antibody** raised against conjugated Aze A.

L13 ANSWER 10 OF 24 MEDLINE
89310515 Document Number: 89310515. PubMed ID: 2746227. Monoclonal anti-conjugated acetylcholine **antibody** and immunohistochemical applications in rat nervous system. **Chagnaud J L**; Souan M L; Charrier M C; **Geffard M**. (Laboratoire d'Immunologie, IBCN-CNRS,

Bordeaux, France.) JOURNAL OF NEUROCHEMISTRY, (1989 Aug) 53 (2) 383-91. Journal code: JAV; 2985190R. ISSN: 0022-3042. Pub. country: United States.
Language: English.

AB Acetylcholine (ACh) conjugates were injected into AKR and DBA mice over a period of 10 weeks. The polyclonal antisera were tested at various immunization times for affinity and specificity using an enzyme-linked immunosorbent assay (ELISA). The most immunoreactive compound was found to be choline-glutaryl-bovine serum **albumin** (or conjugated ACh). The AKR and DBA mice yielding the highest apparent affinity were killed, and the spleen cells were fused with X63 or SP2/O/Ag mouse myeloma cells. Supernatants of confluent cultures were tested for the presence of anti-conjugated ACh **antibodies** using the same ELISA method. The best results were obtained with the hybridomas from AKR spleen cells and X63 mouse myeloma cells. Monoclonal **antibody** affinity and specificity were then evaluated by a radioimmunological procedure using iodinated monoclonal anti-conjugated ACh **antibody**. From competition experiments, the most immunoreactive compound was choline-glutaryl-protein. The other related compounds were recognized either poorly or not at all. The high affinity and specificity of our monoclonal **antibody** enabled us to visualize ACh molecules on fixed rat brain sections. ACh was fixed with a mixture of nitrobenzyl alcohol and glutaraldehyde. Many ACh-immunoreactive cell bodies and fibers were seen on sections from the basal forebrain and spinal cord. Preadsorption and other immunohistochemical tests demonstrated that the ACh staining was highly specific.

L13 ANSWER 11 OF 24 MEDLINE

89208132 Document Number: 89208132. PubMed ID: 2565132. Monoclonal **antibody** directed against glutaraldehyde conjugated glutamate and immunocytochemical applications in the rat brain. **Chagnaud J L;** Campistron G; **Geffard M.** (Laboratoire d'immunologie, IBCN-CNRS, Universite de Bordeaux II, France.) BRAIN RESEARCH, (1989 Feb 27) 481 (1) 175-80. Journal code: B5L; 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB Like other small-sized neurotransmitter molecules, glutamate (Glu) was conjugated to carrier proteins via glutaraldehyde (G). Human serum **albumin** (HSA) and thyroglobulin (TH) conjugates were alternately injected into mice. When a relevant immune response was obtained for **antibody** affinity and specificity, hybridization of spleen activated lymphocytes with SP2/O/Ag myeloma cells was performed. Supernatant culture media of hybridomas were tested for the presence of anti-conjugated Glu **antibodies** with our ELISA method. Selected hybridomas giving good **antibody** affinity and specificity were then cloned by the limiting dilution technique. Using DEAE-chromatographed ascites fluid, Glu reactivity was observed on the cortex and the hippocampus. Staining obtained with this monoclonal **antibody** was in agreement with that observed with previous polyclonal antisera directed against conjugated Glu or monoclonal anti-gamma-glutamyl-Glu **antibody**.

L13 ANSWER 12 OF 24 MEDLINE

89175424 Document Number: 89175424. PubMed ID: 2925840. Identification

and characterization of anti-conjugated azelaic acid **antibodies** in multiple sclerosis. Daverat P; **Geffard M**; Orgogozo J M. (Centre Hospitalier Universitaire, Bordeaux, France.) JOURNAL OF NEUROIMMUNOLOGY, (1989 Apr) 22 (2) 129-34. Journal code: HSO; 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB Human sera from patients with multiple sclerosis (MS) were tested using an enzyme-linked immunosorbent assay (ELISA) method on well plates coated with various dicarboxylic acid (C4 to C10) protein conjugates. Specific immunological binding was found with an azelaic acid (AzeA, C9) conjugate.

The **antibody** titer was higher in the sera from the patients in acute relapse than with the progressive form, and higher than that from sera of patients with other neurological diseases and healthy subjects. Modifications of coating concentrations and of **antibody** dilutions, and experiments with preadsorption enabled determination of binding specificity. Competition experiments with related conjugates demonstrated that the AzeA residue was 167 times better recognized by **antibodies** from MS patients in acute relapse than those from controls. The suberic and sebasic acid conjugates which only differ from the AzeA conjugate by one methylene group were less well-recognized by MS sera (11 and 47 times, respectively) than the conjugate AzeA-BSA.

L13 ANSWER 13 OF 24 MEDLINE

89287744 Document Number: 89287744. PubMed ID: 3150819. Visualization of L-dihydroxyphenylalanine in rat brain by using specific **antibodies**. Mons N; Danel N; **Geffard M**. (Laboratoire de Neuroimmunologie, IBCN-CNRS, Universite de Bordeaux II, France.) BRAIN RESEARCH, (1988 Jun 7) 451 (1-2) 403-7. Journal code: B5L; 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB L-Dihydroxyphenylalanine (L-DOPA) was conjugated to different protein carriers with glutaraldehyde (G). During the synthesis of the catecholamine conjugates, precautions were taken in order to preserve the structure of L-DOPA. Reduced and non-reduced conjugates were injected to rabbits according to a specific immunization protocol. Anti-L-DOPA **antibody** affinity and specificity were evaluated by using ELISA tests. The most immunoreactive compounds were the non-reduced conjugate, L-DOPA = G = BSA and the reduced one, L-DOPA-G-BSA. The other conjugated catecholamines were poorly recognized or not at all. These antisera enabled us to specifically visualize the precursor of the catecholaminergic neurotransmitters which are: dopamine, noradrenaline and adrenaline in the G-fixed rat brains.

L13 ANSWER 14 OF 24 MEDLINE

87197317 Document Number: 87197317. PubMed ID: 3106574. Specific antisera against the catecholamines: L-3,4-dihydroxyphenylalanine, dopamine, noradrenaline, and octopamine tested by an enzyme-linked immunosorbent assay. Mons N; **Geffard M**. JOURNAL OF NEUROCHEMISTRY, (1987 Jun) 48 (6) 1826-33. Journal code: JAV; 2985190R. ISSN: 0022-3042. Pub. country: United States. Language: English.

AB Antisera were raised against L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine (DA), noradrenaline (NA), and octopamine (OA). This was achieved by coupling each molecule to bovine serum **albumin** or human serum **albumin** using glutaraldehyde. The conjugated aromatic amines were kept in a reducing medium containing sodium metabisulfite. Antiserum specificity was tested using an enzyme-linked immunosorbent assay method

for catecholamines. Competition experiments were done between the immunogen coated on the well plates and each catecholamine, either in the free state or in conjugated form, previously incubated with an antiserum. In each case, the nonconjugated compound was poorly recognized. The nonreduced conjugates of L-DOPA and DA were well recognized, whereas those of NA and OA were poorly immunoreactive. The cross-reactivity ratios established in the competition experiments allowed the specificity of the immune response to be defined. In each case, it was found to be high. The results suggest that the **antibodies** of L-DOPA and DA antisera recognize preferentially the catechol moiety, whereas for the anti-NA and anti-OA **antibodies**, the lateral chain is important.

L13 ANSWER 15 OF 24 MEDLINE

88075612 Document Number: 88075612. PubMed ID: 3479933. Anti-acetylcholine **antibodies** and the pathogenesis of myasthenia gravis. Souan M L; **Geffard M**; Vieillemaringe J; Lebrun-Grandie P; Orgogozo J M. (Laboratoire de Neuroimmunologie, IBCN-CNRS, Bordeaux, France.) ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1987) 505 423-38. Journal code: 5NM; 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB Using an ELISA system, **antibodies** recognizing conjugated acetylcholine (ACh) were detected in sera of patients suffering from myasthenia gravis. The mean **antibody** level was three times higher in sera from myasthenic than from control patients. No correlation was found between anti-ACh **antibody** levels and anti-ACh receptor (AChR) titer. Also, the anti-ACh **antibody** titers were independent of sex and age of patients. Competition experiments demonstrated that the most immunoreactive compounds were choline-glutaryl-bovine serum **albumin** (BSA) and choline-succinyl-BSA. **Antibodies** present in the sera of myasthenic patients recognized an antigenic determinant mimicking conjugated ACh. The **antibody** affinity and specificity were sufficiently high for the detection of ACh in locust brain.

L13 ANSWER 16 OF 24 MEDLINE

88079342 Document Number: 88079342. PubMed ID: 3121135. First characterization of 5-hydroxytryptophan in rat brain by using specific **antibodies**. **Geffard M**; Touret M; Kitahama K. (Laboratoire de Neuroimmunologie, IBCN-C.N.R.S., Universite de Bordeaux II, France.) BRAIN RESEARCH, (1987 Nov 17) 426 (1) 191-6. Journal code: B5L; 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB DL-5-Hydroxytryptophan (5-HTP) was conjugated to bovine serum **albumin** and human serum **albumin** with glutaraldehyde (G). These conjugates made it possible to raise specific antisera in two rabbits. Their specificity and affinity were evaluated using an enzyme-linked immunosorbent assay and immunocytochemistry. For two antisera obtained, the most immunoreactive antigen was 5-HTP-G-protein, indicating that the same immune response was developed. The other conjugated indoleamines (5-methoxytryptophan-G-protein, tryptophan-G-protein) were poorly recognized or not at all (5-methoxytryptamine-G-protein, serotonin-G-protein, tryptamine-G-protein). These 5-HTP antisera enabled us to specifically visualize the precursor of serotonin in the raphe nuclei of G-fixed rat brains.

L13 ANSWER 17 OF 24 MEDLINE

86245002 Document Number: 86245002. PubMed ID: 3719676. The dopaminergic

innervation of the goldfish pituitary. An immunocytochemical study at the electron-microscope level using **antibodies** against dopamine. Kah O; Dubourg P; Onteniente B; **Geffard M**; Calas A. CELL AND TISSUE RESEARCH, (1986) 244 (3) 577-82. Journal code: CQD; 0417625. ISSN: 0302-766X. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB The dopaminergic innervation of the goldfish pituitary gland was studied by immunocytochemistry at the electron-microscope level using highly specific **antibodies** against dopamine coupled to bovine serum **albumin** with glutaraldehyde. A satisfactory preservation of the tissue was achieved after immersion in 5% glutaraldehyde in phosphate buffer containing sodium metabisulfite to prevent oxidation of the endogenous dopamine. The immunocytochemical procedure was performed on Vibratome sections using the preembedding method. Immunoreactivity was restricted to part of the neurosecretory type-B fibers (diameter of the secretory vesicles lower than 100 nm) in which it was found to occupy the whole cytoplasm. Labeled fibers were observed within the neurohypophysis in the different parts of the gland and in the adenohypophyseal tissue where immunoreactive profiles were detected in close apposition to the different cell types. These data are in agreement with previous results obtained by means of radioautography and further support a role for dopamine in the neuroendocrine regulation of pituitary functions in teleosts.

L13 ANSWER 18 OF 24 CAPLUS COPYRIGHT 2001 ACS

1986:205413 Document No. 104:205413 **Antibodies** directed against a haptenic group containing a choline moiety. **Geffard, Michel** (Centre National de la Recherche Scientifique, Fr.). Fr. Demande FR 2561660 A1 19850927, 22 pp. (French). CODEN: FRXXBL. APPLICATION: FR 1984-4663 19840326.

AB **Antibodies** are prepd. that are directed against a hapten contg. a choline moiety. These **antibodies** may be useful for detecting acetylcholine or choline esters. Thus, bovine serum **albumin** (BSA) was reacted with glutaric acid anhydride (AG). The product was reacted with choline to yield an immunogen of the formula [(CH3)3N+CH2CH2OCO(CH2)3CO]x-[M] (where 70 < x < 120, and M = BSA). Rabbits were immunized with this immunogen and other immunogens in which M = human serum **albumin** and Hb.

L13 ANSWER 19 OF 24 CAPLUS COPYRIGHT 2001 ACS

1985:558990 Document No. 103:158990 **Antibodies** capable of specifically recognizing haptenic groups, and their application, and antigens for the preparation of these **antibodies**. **Geffard, Michel** (Centre National de la Recherche Scientifique, Fr.; Institut National de la Sante et de la Recherche Medicale (INSERM)). Eur. Pat. Appl. EP 149405 A2 19850724, 26 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (French). CODEN: EPXXDW. APPLICATION: EP 1984-402762 19841228. PRIORITY: FR 1983-21087 19831230.

AB New monoclonal **antibodies** are produced which are capable of reacting with haptenic groups of the formula: -NH-(CH2)n-NH-hapten, where n is a no. between 4 and 6 and the hapten is the remnant of a mol. of the formula hapten-NH2 or hapten=NH. Antigens used to induce formation of these **antibodies** and the use of the **antibodies** in the detn. or detection of haptens (e.g., hormones, amino acids, oligopeptides, vitamins, drugs, toxins, and neuromodulators) in soln. or in tissue cell cultures are described. Thus, **antibodies** to catecholamines were

prepd. by using dopamine (DA)-conjugated bovine serum **albumin** (BSA) as the immunogen in rabbits. Radioactive ligands were prepd. by conjugating [3H]DA with N-.alpha.-acetyl-L-lysine N-methylamide (ALM) by means of glutaraldehyde ([3H]DA-G-ALM), and the **antibody** titer, specificity, and affinity were detd. using a competitive immunoassay with the radioactive ligand and a serum sample. **Antibody** to [3H]DA-G-ALM was inhibited the most by 10-9 to 10-7M DA-G-ALM, but was not by 10-6M DA. The Kd for anti-DA was 1.48 .times. 10-8M and its affinity const. for DA-G-ALM was 6.7 .times. 107 L/mol.

L13 ANSWER 20 OF 24 MEDLINE

85133700 Document Number: 85133700. PubMed ID: 3919158. Antisera against the indolealkylamines: tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-methoxytryptophan, and 5-methoxytryptamine tested by an enzyme-linked immunosorbent assay method. **Geffard M;** Dulluc J; Rock A M. JOURNAL OF NEUROCHEMISTRY, (1985 Apr) 44 (4) 1221-8. Journal code: JAV; 2985190R. ISSN: 0022-3042. Pub. country: United States.
Language: English.

AB Antisera were raised against tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-methoxytryptophan, and 5-methoxytryptamine, by conjugating each molecule to bovine serum **albumin** and to human serum **albumin** via glutaraldehyde, in such a way as to preserve the original part. **Antibody** specificity was tested with the enzyme-linked immunosorbent assay method. The specificity of each anti-indolealkylamine-glutaraldehyde **antibody** was established with competition experiments by using an adsorbed immunogenic conjugate and indolealkylamines either free or conjugated with poly-L-lysine. The nonconjugated compounds were poorly recognized. In the same way, the nonreduced conjugates always appeared less immunoreactive than the reduced ones. Calculated from the specificity study of each antiserum, the cross-reactivity ratios were found to be smallest for the most immunoreactive conjugates. Thus, a specific immune response was defined for each compound belonging to the same metabolic pathway.

L13 ANSWER 21 OF 24 MEDLINE

84215059 Document Number: 84215059. PubMed ID: 6427408. **Antibodies** to dopamine: radioimmunological study of specificity in relation to immunocytochemistry. **Geffard M;** Kah O; Onteniente B; Seguela P; Le Moal M; Delaage M. JOURNAL OF NEUROCHEMISTRY, (1984 Jun) 42 (6) 1593-9. Journal code: JAV; 2985190R. ISSN: 0022-3042. Pub. country: United States. Language: English.

AB Two classes of anti-3,4- dihydroxyphenylethylamine (dopamine) **antibodies** were raised in rabbits using dopamine conjugated to **albumin** either via formaldehyde or via glutaraldehyde. Each was usable for immunohistochemical detection of dopamine neurons provided that the tissue was fixed by the homologous cross-linking agent. However, anti-dopamine-glutaraldehyde **antibodies** turned out to be of more general use because of the better fixative properties of glutaraldehyde which fixed dopamine in rat and in teleost, whereas formaldehyde only worked in lower vertebrates (such as goldfish) and not in rat brain. The specificity of anti-dopamine-glutaraldehyde **antibodies** was firmly established by competition experiments in equilibrium dialysis, using an immunoreactive tritiated derivative synthesized by coupling dopamine to N-alpha-acetyl-L-lysine N-methylamide via glutaraldehyde.

Specificity studies in vitro and immunohistological results demonstrating the specific staining of dopaminergic neurons were found to correlate well.

L13 ANSWER 22 OF 24 MEDLINE

84270348 Document Number: 84270348. PubMed ID: 6431267. Antisera against catecholamines: specificity studies and physicochemical data for anti-dopamine and anti-p-tyramine **antibodies**. **Geffard M** ; Seguela P; Heinrich-Rock A M. MOLECULAR IMMUNOLOGY, (1984 Jun) 21 (6) 515-22. Journal code: NG1; 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Antibodies** against dopamine and p-tyramine were raised in rabbits. The two catecholamines were conjugated to **albumin** by glutaraldehyde. The specificity of the **antibodies** was established by equilibrium dialysis competition experiments using an immunoreactive tritiated derivative synthesized by coupling dopamine or p-tyramine to N-alpha-acetyl-L-lysine N-methylamide with glutaraldehyde. Hence, these radiolabelled ligands mimicked the antigenic determinant of conjugated immunogens. A comparison of the data obtained showed the high specificity of each antiserum for its hapten coupled by glutaraldehyde. The anti-dopamine **antibodies** recognized dopamine-glutaraldehyde but not p-tyramine-glutaraldehyde. The opposite occurred for the anti-p-tyramine **antibodies**. A slight modification of the molecular structure provided the opportunity for a specific response against that molecule. But this difference was more important when related to the hapten region where the **antibody** affinity was maximal. The cross-reactivity was observed to be more important dopamine and p-tyramine than between dopamine and noradrenaline on the one hand and between p-tyramine and dopamine than p-tyramine and octopamine on the other hand.

L13 ANSWER 23 OF 24 MEDLINE

84129539 Document Number: 84129539. PubMed ID: 6697233. First demonstration of highly specific and sensitive **antibodies** against dopamine. **Geffard M**; Buijs R M; Seguela P; Pool C W; Le Moal M. BRAIN RESEARCH, (1984 Feb 27) 294 (1) 161-5. Journal code: B5L; 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB Dopamine was coupled to bovine serum **albumin** (BSA) with glutaraldehyde, precautions were taken in order to preserve the catechol ring. After injection of this immunogen into rabbits, anti-dopamine **antibodies** were obtained and tested using radioimmunochemical binding studies and adsorption to catecholamine covered sepharose beads. A good correlation was found between the results of the different test systems, allowing us to visualize dopamine specifically in glutaraldehyde-fixed rat brains.

L13 ANSWER 24 OF 24 MEDLINE

83179963 Document Number: 83179963. PubMed ID: 6820314. [1st immunocytochemical application of an anti-dopamine **antibody** in the study of the central nervous system]. Premiere application immunocytochimique d'un anticorps antidopamine a l'etude du systeme nerveux central. **Geffard M**; Kah O; Chambolle P; Le Moal M; Delaage M. COMPTES RENDUS DES SEANCES DE L ACADEMIE DES SCIENCES. SERIE III, SCIENCES DE LA VIE, (1982 Dec 20) 295 (13) 797-802. Journal code: CA4; 8108553. ISSN: 0249-6313. Pub. country: France. Language: French.

AB An **antibody** against dopamine was raised in Rabbits by means of

an immunogen constituting dopamine coupled to bovine serum **albumin** by formaldehyde. These **antibodies** were applied to frozen sections of Goldfish (Carassius auratus) brain fixed by 4% paraformaldehyde. The localisation of immunoreactive structures corresponded to regions rich in dopamine detected by histofluorescence. The specificity of this antiserum tested with the aid of dopamine analogues coupled with a carrying protein different from that of the **antibody**, indicates its validity as a method for the detection of dopaminergic neurons.

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